SYNTHESIS OF (±)-[1,1'-$^{15}N_2$, 2'-^{13}C]-*trans*-3'-METHYLNICOTINE

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Summary

The synthesis of(±)-[1,1'-$^{15}N_2$, 2'-^{13}C]-*trans*-3'-methylnicotine is reported. ^{15}N-3-Bromopyridine obtained from bromination of pyridine was formylated with nBuLi/[carbonyl-^{13}C]-methyl formate. The resulting ^{15}N-Pyridine-3-[^{13}C-carbonyl]-carboxaldehyde was reacted with ^{15}N-methylamine and then the resulting Schiff's base was condensed with succinic anhydride to give (±)-[1,1'-$^{15}N_2$, 5'-^{13}C]-*trans*-4'-carboxycotinine. Reduction with lithium aluminum hydride and mesylation followed by reduction with Zn/NaI gave (±)-[1,1'-$^{15}N_2$, 2'-^{13}C]-*trans*-3'-methylnicotine.

Key words
(±)-[1,1'-$^{15}N_2$, 5'-^{13}C]-*trans*-4'-Carboxycotinine, (±)-[1,1'-$^{15}N_2$, 2'-^{13}C]-*trans*-3'-Hydroxymethylnicotine, (±)-[1,1'-$^{15}N_2$, 2'-^{13}C]-*trans*-3'-Mesyloxymethylnicotine, (±)-[1,1'-$^{15}N_2$, 2'-^{13}C]-*trans*-3'-Methylnicotine Cotinine, Passive Smoking.

INTRODUCTION

An accurate quantitative measurement of passive exposure to smoke has been a subject of intense study because of its significance in improving the validity of the results of epidemiologic studies related to passive smoking (1). Cotinine, a major metabolite of nicotine, has a relatively longer metabolic half life than nicotine and, as such, represents an intake marker of choice of exposure of nonsmokers to nicotine in environmental tobacco smoke (1a).

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In the course of developing a sensitive and accurate high resolution gas chromatography (HRGC)/high resolution mass spectrometry (HRMS) method for measuring cotinine in the serum of a representative sample of the U.S. population (2), we recognized the need for a [$^{15}N_2$, ^{13}C]-triple-labeled-methylnicotine as a standard for isotope dilution mass spectrometry. We chose this molecule as an external standard for evaluating the recovery of cotinine in biological samples and for estimating the resolving power of the high resolution mass spectrometer (2). The internal standard used in our HRGC/HRMS method to quantify cotinine in serum is a $^2H_3$-labeled cotinine (2). In order to validate that the analysis was conducted at high resolution, the resolving power (RP) of the mass spectrometer must be assessed at the time of the measurement. The difference in molecular mass between the internal standard $^2H_3$-cotinine (179.1138) and the [$^{15}N_2$,^{13}C]-methylnicotine (179.1288) is exactly right to make this assessment. Documenting the high resolution measurement process provides a high degree of confidence in the measurement of cotinine in the representative sample of the U.S. population (2). Although several ^{15}N and ^{13}C-labeled nicotine and cotinine derivatives have been synthesized before (3), synthesis of triple-labeled methylnicotine had not been reported. We now report the synthesis of (±)-[1,1'-$^{15}N_2$, 2'-^{13}C]-*trans*-3'-methylnicotine (Figure 1) partly based on the method of Cushman and Castagnoli (4).

EXPERIMENTAL

Materials: ^{15}N-pyridine, (carbonyl-^{13}C)-methyl formate, and ^{15}N-methylamine in benzene (87.5 mg/mL) were obtained from Cambridge Isotopes (Woburn, Massachusetts). Succinic anhydride, methane sulfonylchloride, bromine, lithium aluminum hydride, sodium iodide and zinc were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin). A sample of 114% sulfuric acid was provided by E. I. du Pont de Nemours

& Co. (Inc.), Chemicals & Pigments Dept (Wilmington, Delaware). All the solvents for extractions and HPLC were from Burdick & Jackson Division (Baxter Healthcare Corporation, Muskegon, Michigan). All chemicals and solvents were used without further purification.

Apparatus: All analytical and preparative HPLC was performed by using a Waters (Milford, Massachusetts) Model 6000A Solvent Delivery System equipped with Dynamax (Rainin Instrument Co., Woburn, Massachusetts) (4.6 mm i.d. x 25 cm L or 21.4 mm i.d. x 25 cm L) silica columns coupled with a Waters (Milford, Massachusetts) Lambda-Max Model 480 UV detector interfaced with a Hewlett-Packard (Avondale, Pennsylvania) Model 3390 integrator. Mass spectra (electron impact (EI), fast atom bombardment mass spectrometry (FAB-MS), and FAB-MS/MS) were recorded on a VG 70-4SE high resolution magnetic sector mass spectrometer (Manchester, England). FAB-MS and FAB-MS/MS were obtained under the following conditions: accelerating voltage, 8 kV; Cesium ion gun; and 1:5 mixture of dithioerythritol and dithiothreitol (DTE/DTT) as the matrix. One microliter of sample was mixed with the matrix on a flat stainless steel probe target. Cesium ions bombarded the sample with energies up to 35 kV minus the 8kV source potential, resulting in an energy of 27 kV at the sample. MS/MS was also performed under these conditions and parameters. We obtained daughter ions by collision induced decomposition (CID) using helium as the collision gas. EI mass spectra were obtained at a source temperature of 250°C, accelerating voltage, 8 kV; an electron energy of 500 microamps at 30 eV. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian (Palo Alto, California) model XL-300 FT NMR spectrometer.

Methods:

^{15}N-3-Bromopyridine (I). Bromination of pyridine was carried out according the method

of den Hertog et al. with minor modifications (5). ^{15}N-Pyridine (0.5 g, 6.2 mmol) was placed in a pressure tube (Ace Glass) and cooled in an ice bath. To this, 114% oleum (2.5 mL) was added dropwise followed by bromine (0.32 mL, 6.2 mmol). The mixture was heated at 130°C for 18 hr in the sealed pressure tube. The resultant mixture was cooled to room temperature and poured onto ice (10-15 g), the pH was adjusted to 8 by using 10 N sodium hydroxide solution, and the resultant material was extracted with methylene chloride (4 x 50 mL). The methylene chloride extract was dried over anhydrous sodium sulfate and concentrated by fractional distillation by using three Vigreux columns (3 x 130 mm) serially connected. ^{15}N-3-Bromopyridine (retention time = 8.05 min) was separated from unreacted pyridine, and traces of dibrominated pyridines by preparative HPLC using 40% methyl acetate containing pentane at a flow rate of 9 mL/min with UV detection at 254 nm. The fractions containing I were combined and concentrated by fractionally distillating off the solvent with the same distillation setup used before to give a pale yellow liquid (0.59 g, 61%). ^{1}H NMR ($CDCl_3$) δ 8.68-8.72 (m, 1H), 8.51-8.57 (m, 1H), 7.81-7.85 (m, 1H), 7.19-7.26 (m, 1H); mass spectrum, m/z (relative intensity): 160(78), 158(M^+, 78), 87(69), 83(53), 79(100), 51(41).

[$^{15}N_2$,(methylidene-^{13}C)]-N-(3-Pyridylmethylidene)methylamine (III). A solution of n-BuLi (3.8 mmol, 1.5 mL of 2.5 M solution in hexanes) was added to a cold (-78°C, dry ice acetone) solution containing ^{15}N-3-bromopyridine (I) (500 mg, 3.2 mmol) in THF (1.5 mL) which was being stirred under Ar. After the mixture was stirred at -78°C for 20 min, (carbonyl-^{13}C)-methyl formate (400mg, 6.6 mmol) was added dropwise. The resulting mixture was stirred at -78°C for 5 min and then at room temperature. The reaction mixture was diluted with water (5 mL), saturated with NaCl, and extracted with methylene chloride (4 x 50 mL). The combined methylene chloride extract was dried over Na_2SO_4

and concentrated by distillation using the same fractionation setup described above. ^{15}N-Pyridine-3-^{13}C-carboxaldehyde (II) (retention time = 12.3 min at flow rate of 12 mL/min) was isolated from the reaction mixture by preparative HPLC using silica gel as the stationary phase and 75% methyl acetate in pentane as the mobile phase (detection at 254 nm). The pyridine-3-carboxaldehyde product (II, 124 mg, 36%) exhibited FAB-MS; 264(M^++DTE/DTT+H), FAB-MS/MS daughter ions of m/z 264; 264(100), 110(40), 85(22). The synthesis of the Schiff's base (III) was accomplished by following a procedure similar to that of Cushman and Castagnoli (4). A solution of ^{15}N-Pyridine-3-^{13}C-carboxaldehyde (II, 124 mg, 1.1 mmol) in benzene (1 mL), ^{15}N-methylamine (44 mg in 0.5 ml of benzene), and molecular sieves (100 mg) was stirred under Ar for 12 hr at room temperature. The reaction mixture was diluted with methylene chloride (15 mL) and filtered through a bed of celite. The solvent was distilled off to give a yellow liquid residue (III, 115 mg, 82%). The product had ^{1}H nmr ($CDCl_3$) δ 8.79-8.84 (m, 1H), 8.55-8.64 (m, 1.5H), 8.02-8.07 (m, 1.5H),7.30-7.34 (m, 1H), 3.31-3.54 (m, 3H); mass spectrum, m/z (relative intensity): 123(M^+, 82), 122(73), 111(59), 110(91), 97(69), 87(100), 83(71), 81(77), 71(82) and was used in the next step without further purification.

(±)-[1,1'-$^{15}N_2$, 5'-^{13}C]-*trans*-4'-Carboxycotinine (IV). The method employed in the synthesis of this compound is a modification of that of Cushman and Castagnoli (4). A solution of III (115 mg, 0.93 mmol) and succinic anhydride (100 mg, 1.0 mmol) in xylene (1.2 mL) was heated at reflux for 24 hr under Ar. The reaction mixture was cooled, diluted with methanol (10 mL) and decolorized with activated carbon. The solvent was removed under reduced pressure to give a yellowish brown residue. NMR indicated the presence of IV (76.2% by NMR) along with II. ^{1}H NMR (D_6-DMSO) δ 8.60-8.66 (m), 7.65-7.75 (m), 7.25-7.35 (m), 4.92 (dd, 1H, J_{13C-H} = 146 Hz, J_{Ha-Hb} = 5.8 Hz), 2.64-276

(m), 2.86-2.94 (m),; mass spectrum, m/z (relative intensity): 223(M^+, 37), 195(45), 178(27), 150(29), 106(57), 101(48), 91(100), 73(51), 56(62), 55(66).

(±)-[1,1'-$^{15}N_2$, 2'-^{13}C]-*trans*-3'-Hydroxymethylnicotine (V). The trans acid (IV) was stirred for 24 hr at room temperature with a solution of $LiAlH_4$ in ether (2 mL, 1M in THF). The reaction mixture was then worked up by adding water (1 mL) and 2N NaOH (2 mL). The product was extracted with methylene chloride (3 x 50 mL), and the combined extract washed with a solution of saturated NaCl (15 mL), dried over sodium sulfate, and concentrated in vacuo. The resultant gold-colored residue showed the presence of one product (V, 73 mg, 40%) and exhibited ^1H NMR (Figure 1 for proton assignments) δ 8.42-8.49 (m), 7.7-7.74(m), 7.23-7.29 (m), 3.5-3.63 (m, 2H, CH_2O), 3.09-3.24 (m, 1.5H, H_a, H_f), 2.75 (m, 0.5H, H_a), 2.06-2.39 (m, 6H, N-CH_3, H_b, H_c, H_e), 1.62-1.8(m, 1H, H_d); FAB-MS; M^++H (196), FAB-MS/MS daughter ions of m/z 196; 196(100), 178(0.5), 164(1.1), 150(0.4), 138(1.0), 120(0.8), 116(1.6), 106(0.4), 81(0.8).

(±)-[$^{15}N_2$, 2'-^{13}C]-*trans*-3'-Methanesulfonylmethylnicotine (VI). This compound was conveniently prepared from the alcohol V by the general procedure of Crossland and Servis (6). A solution of V (52 mg, 0.3 mmol) in methylene chloride (1.5 mL) containing triethylamine (0.05 mL) was treated with methanesulfonyl chloride (mesyl chloride, 0.03 mL) over a period of 1-2 min. After further stirring for 30 min, the reaction mixture was diluted with methylene chloride (50 mL) and washed with ice water (10 mL) followed by a saturated sodium bicarbonate solution (10 mL) and a salt solution (10 mL). The methylene chloride extract was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The product VI obtained (82 mg, 100%) in this manner was >97% pure by HPLC and used without further purification. ^1H NMR

($CDCL_3$) 8.48-8.56 (m, 2H), 7.71-7.74 (m, 1H), 7.24-7.29 (m, 1H), 4.13-4.16 (m, 2H, CH_2OMs), 3.90(s, 3H, $-SO_3-CH_3$); FAB-MS M^+ (274); FAB-MS/MS daughter ions of m/z 274; 274(100), 216(0.2), 194(0.3), 178(0.7), 164(0.3),

(±)-[1,1'-$^{15}N_2$, 2'-^{13}C]-*trans*-3'-Methylnicotine (VII). The mesyloxy group in VI was reductively removed to prepare VII by a general procedure described by Fujimoto and Tatsuno (7). A suspension of VI (82 mg, 0.3 mmol), sodium iodide (225 mg), and zinc powder (190 mg) in 1,2-dimethoxyethane:DMSO (4:1, 1.5 mL) was refluxed for 3 hr with stirring. The reaction mixture was then decomposed with water (10 mL) and extracted with methylene chloride. The methylene chloride phase was washed with a saturated sodium chloride solution, dried over sodium sulfate, and concentrated in vacuo to give VII (28 mg, 52%). The product (VII, retention time = 12.3 min) was isolated by HPLC using an amino bonded silica column using 25% ethyl acetate in hexane as the mobile phase at a flow rate 1.5 mL/min with the detection at 254 nm. ^1H NMR (see Figure 1 for proton assignments) δ 8.46-8.33 (m, 2H), 7.65-7.67 (m, 1H), 7.23-7.27 (m, 1H), 3.15-3.27 (m, 1H, H_f), 2.73-2.78 (m, 1H, H_a), 2.35-2.42(q, 1H), 1.98-2.3(m, 5H, NCH_3, H_c, H_e), 1.4-1.52(m, 1H, H_d), 0.91 (dd, 3H); FAB-MS;(M^++H) (180), FAB-MS/MS daughter ions of m/z-180; 180(100), 165(0.2), 162(0.2), 150(0.2), 148(0.5), 138(0.4), 136(1.9).

RESULTS AND DISCUSSION

Our ongoing research on developing a sensitive and accurate HRGC/HRMS method to measure cotinine in biological fluids (2) prompted us to undertake the synthesis of [1,1'-$^{15}N_2$, 2'-^{13}C]-3'-methylnicotine. Although unlabeled 3'-methylnicotine had been prepared from commercially available pyridine-3-carboxaldehyde (4), the synthesis of the

compound of interest had to be carried out using commercially available ^{15}N-pyridine, [carbonyl-^{13}C]-methyl formate and ^{15}N-methylamine as building blocks. Our procedure for synthesizing triple-labeled-3'-methylnicotine is a modification of the method described for unlabeled 3'-methyl nicotine and is outlined in Figure 1. The first reaction step involves bromination of pyridine by a method described by den Hertog et al. (5). The yield of ^{15}N-3-bromopyridine (I) was about 61% because of incomplete reaction and byproduct formation. To minimize the loss of ^{15}N-3-bromopyridine during solvent removal, solvent was fractionally distilled at atmospheric pressure using three Vigreux columns serially connected. Lithiation of ^{15}N-3-bromopyridine at -78°C followed by reaction with (carbonyl-^{13}C)-methyl formate yielded ^{15}N-pyridine-3-(carbonyl-^{13}C)-carboxaldehyde (II) in 36% yield. As reported by Castagnoli (8), [$^{15}N_2$,(methylidene- ^{13}C)]-N-(3-pyridylmethylidene)methylamine (III) was condensed with succinic anhydride in refluxing xylene to give the trans isomer of [1,1'-$^{15}N_2$, 5'-^{13}C]-4'-carboxycotinine (IV). The transformation of the carboxy group to a methyl group was undertaken by first reducing it with lithium aluminum hydride to give the primary alcohol (V). Although attempts to form the tosyl ester from V resulted in incomplete conversion, methanesulfonyl chloride successfully converted the alcohol to VI in quantitative yield and is attributed to the fact that methanesulfonyl chloride has a smaller steric requirement than the tosyl derivative. Although lithium aluminum hydride (LAH) is widely used in reducing sulfonate esters to the corresponding alkane (9), our attempts to reduce VI to VII using this reagent resulted in a complex mixture of products. The LAH reduction is known to give undesired elimination products and the parent alcohol as well as the desired alkane for certain alcohols (10). As a result of our attempts to seek an alternate route for reducing VI to VII, reduction with NaI/Zn in glyme under reflux was found to convert VI to VII (52%). This reaction presumably proceeds via formation of the corresponding

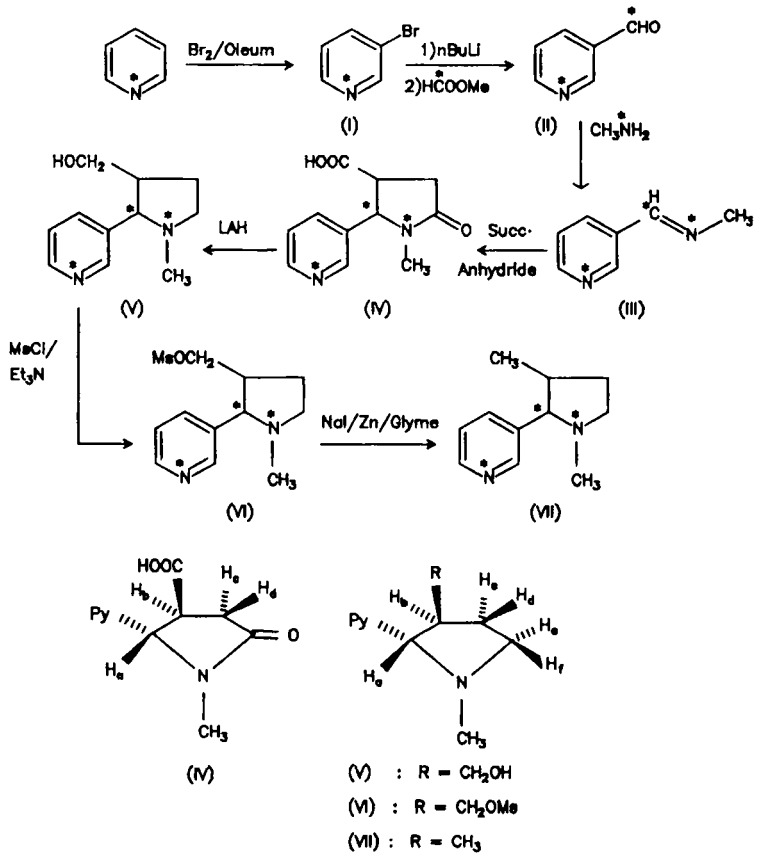

Figure 1

iodide and the corresponding organo zinc intermediate. HPLC analysis of the final product indicated that the product is about 96% pure. The chemical shift assignments for our final product and some of our intermediates were based on a detailed study reported by Cushman and Castagnoli for *trans*-3'-methylnicotine. They made their NMR assignments using model deuteration and pseudocontact shift reagent studies (4). Since labeled pyridine-3-carboxaldehydes are not commercially available, this method represents a general method for synthesizing labeled-nicotine derivatives.

The labeled methylnicotine (VII) is currently being used in our isotope-dilution HRGC/HRMS method for analyzing human serum for cotinine (2). The method involves

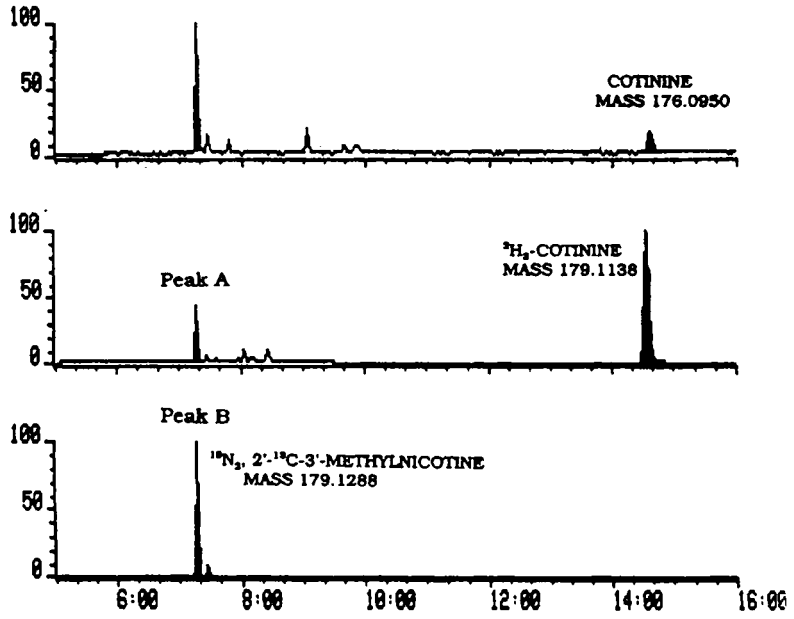

Figure 2

spiking one gram of serum with $^{2}H_{3}$-labeled cotinine as the internal standard used for quantification followed by a multistep sample cleanup (2). The mass spectral area count ratio of native cotinine in the sample to the internal standard is then used to quantify the native cotinine by comparing it with a standard curve developed by using accurately weighed analytical standards. The labeled-methylnicotine (VII) is added to the sample extract just before mass spectral analysis and is used to calculate the percentage of recovery of the internal standard through the multistep cleanup procedure as well as to ensure that the mass spectrometer remained at 10,000 resolving power (RP) during the sample analysis. The mass spectral RP necessary to separate $^{2}H_{3}$-cotinine (m/z 179.1138) from VII (m/z 179.1288) is 11,950. Consequently, if the MS is set at lower than 11,950 RP, these two masses will not be completely resolved (Figure 2). The MS is tuned to 10,000 RP at the beginning of each day, and analytical standards are analyzed. With time, a quality control (QC) chart can be constructed for the ratio of peak A to peak B (Figure

2). This ratio is measured for each unknown serum sample in our studies, and when the ratio is within the QC limits, it provides a high degree of confidence that the measurement was conducted under high resolution (10,000 RP) conditions.

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DISCLAIMER

Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

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